

## **Supporting Information**

### **Highly Stabilized $\alpha$ -Helical Coiled Coils Kill Gram-Negative Bacteria by Multicomplementary Mechanisms under Acidic Condition**

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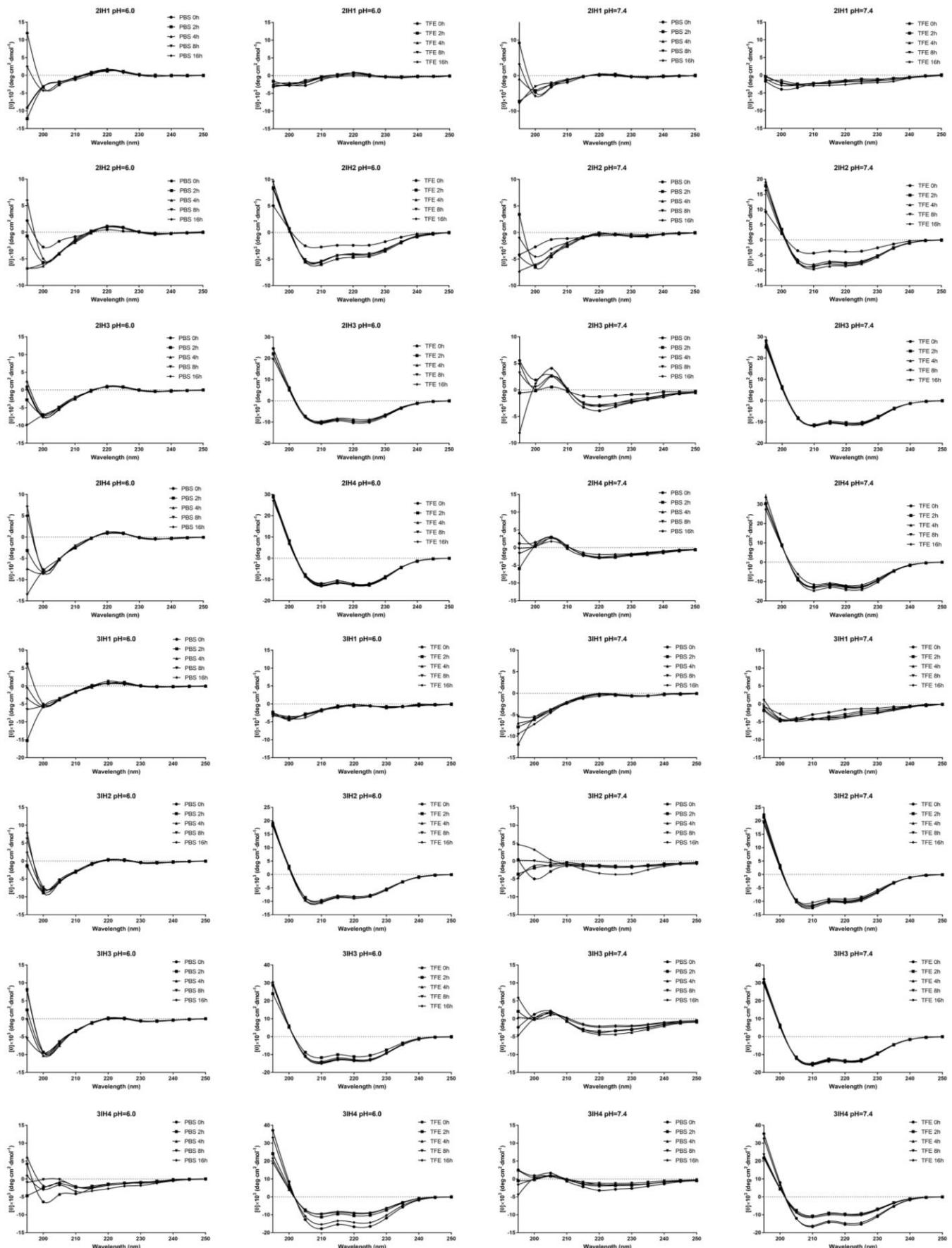
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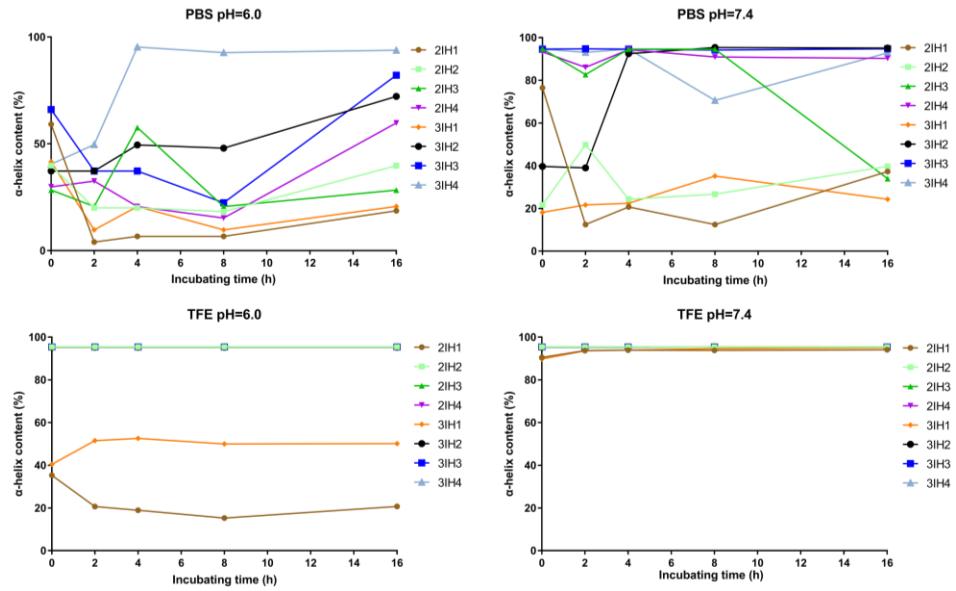
‡ These authors contributed equally to this work.

## Supporting Figures

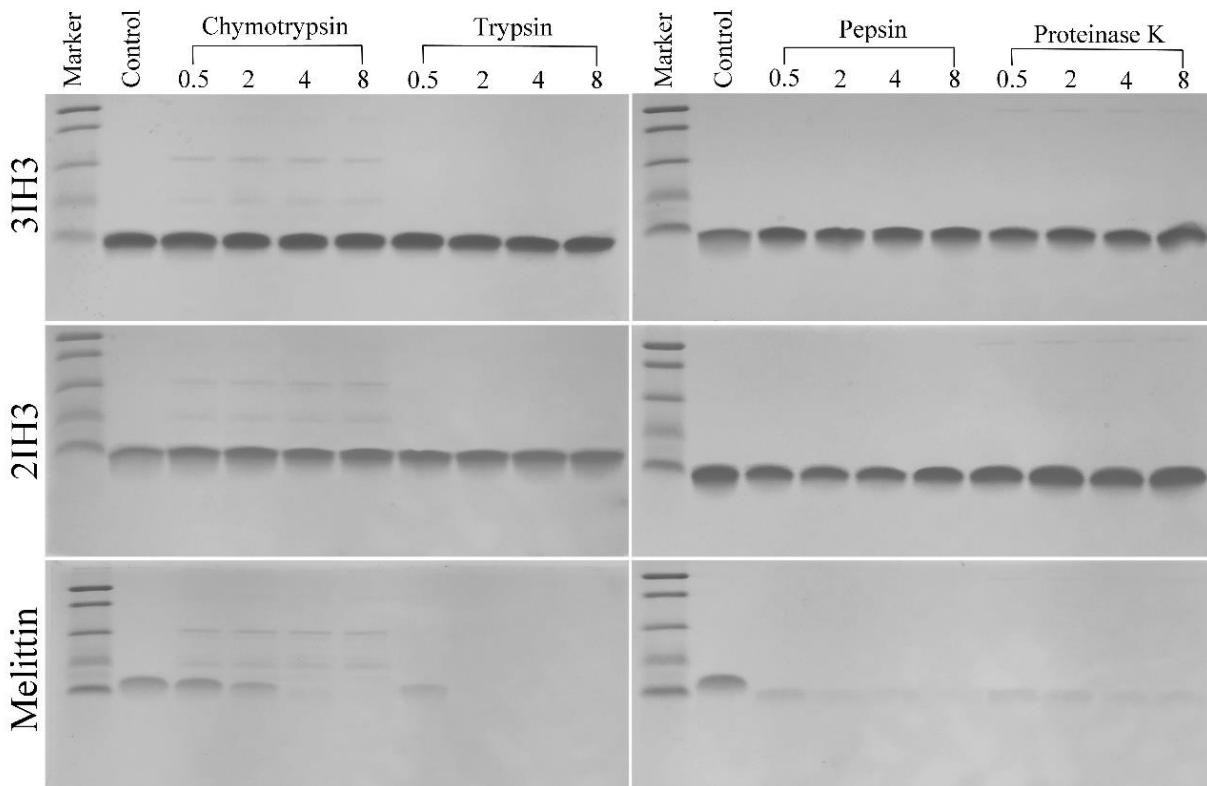


**Figure S1.** The CD spectra of  $\alpha$ -helical coiled coil peptides after incubation with 10 mM PBS (pH = 6.0 or 7.4) or

50% TFE (pH = 6.0 or 7.4) for 0, 2, 4, 8 and 16h. The CD spectra were measured at room temperature with a J-820 spectropolarimeter (Jasco, Japan). The CD spectra were smoothed by GraphPad Prism 7.



**Figure S2.** The quantitative degradation (in percentage) of the  $\alpha$ -helical coiled coil peptides vs incubation time at different pH conditions. The data was estimated online using CD spectral ( $\lambda_{195-240\text{nm}}$ ) by K2D3 (<http://cbdm-01.zdv.uni-mainz.de/~andrade/k2d3//>).



**Figure S3.** Cleavage of the designed peptides by proteases. Each peptide was incubated with chymotrypsin,

trypsin, pepsin and proteinase K in the digestion buffer at 37 °C. At 0.5, 2, 4 and 8 h, the digestion mixture was sampled and analyzed by 16.5% tricine–SDS–PAGE. The molecular weight of the marker from top to bottom corresponds to 31, 20.1, 14.4, 6.5 and 3.3 kDa, respectively.

## Supporting Tables

**Table S1.** The MICs ( $\mu$ M) of the  $\alpha$ -helical coiled coil peptides against Gram-negative bacteria at pH 7.4<sup>a</sup>.

Peptides	<i>E. coli</i>	<i>P.</i>	<i>P.</i>	<i>S.</i>	<i>S.</i>					
	25922	UB1005	K88	K99	078	987P	27853	PAO1	14028	7731
2IH1	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
2IH2	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
2IH3	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
2IH4	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3IH1	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3IH2	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3IH3	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
3IH4	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64

<sup>a</sup> Minimum inhibitory concentration ( $\mu$ M) is defined as the minimum concentration of an antimicrobial agent at which no visible microbial growth is observed. Data are representative of three independent experiments.

**Table S2.** The MICs ( $\mu$ M) of the  $\alpha$ -helical coiled coil peptides against Gram-positive bacteria at pH 7.4 or 6.0<sup>a</sup>.

Peptides	pH = 7.4			pH = 6.0		
	<i>S. aureus</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. aureus</i>	<i>S. aureus</i>	<i>S. epidermidis</i>
	25923	29213	12228	25923	29213	12228
2IH1	>64	>64	>64	>64	>64	>64
2IH2	>64	>64	>64	>64	>64	>64
2IH3	>64	>64	>64	>64	>64	>64
2IH4	>64	>64	>64	>64	>64	>64
3IH1	>64	>64	>64	>64	>64	>64
3IH2	>64	>64	>64	>64	>64	>64
3IH3	>64	>64	>64	>64	>64	>64
3IH4	>64	>64	>64	>64	>64	>64

<sup>a</sup> Minimum inhibitory concentration ( $\mu$ M) is defined as the minimum concentration of an antimicrobial agent at which no visible microbial growth is observed. Data are representative of three independent experiments.

**Table S3.** The MICs ( $\mu$ M) of the peptides against fungi and probiotics at pH 6.0<sup>a</sup>

Peptides	Fungi			Probiotics	
	<i>C. albicans</i>	<i>C. tropicalis</i>	<i>C. parapsilosis</i>	<i>L. rhamnosus</i>	<i>L. rhamnosus</i>
	2.2086	2.1975	2.3989	8014	7469
2IH1	>64	>64	>64	>64	>64
2IH2	16	1	2	>64	>64
2IH3	1	0.5	1	64	>64
2IH4	2	1	2	8	64
3IH1	>64	>64	>64	>64	>64
3IH2	8	1	4	>64	>64
3IH3	2	1	2	64	>64
3IH4	>64	8	64	2	64
Melittin	8	1	8	1	2

<sup>a</sup>Minimum inhibitory concentration ( $\mu$ M) is defined as the minimum concentration of an antimicrobial agent at which no visible microbial growth is observed. Data are representative of three independent experiments.